GDR2588
Microscopie et Imagerie du Vivant

6th Biosensor Meeting
Orsay, 12-13 May 2016

Bâtiment des colloques (338)
Faculty of Sciences, Paris-Sud University

Invited speakers

Carsten Grashoff, MPI Biochemistry, Munich (Germany)
Ranieri Bizzarri, Nanoscience Institute, Pisa (Italy)

Sessions

Mechanical sensors, cell migration and polarisation
Biosensors, chemistry & probes
Biosensors for cell signaling : applications and recent developments

Meeting Objectives

Promoting the development and implementation of real time biosensor imaging approaches to decipher the spatio-temporal dynamics and underlying regulatory mechanisms of signaling cascades, kinase activities, protein-protein interactions and more... from single cells to complex biological systems including tissues, whole embryos and adult animals.
The 6th edition will include an special focus on mechanical sensors.

Organizing Committee

Olivier Gavet (Villejuif), Fabienne Mérola (Orsay), May Morris (Montpellier), Clotilde Randriamampita (Paris), Ève Ranvier (Orsay), Franck Riquet (Gand), Grégoire Vandecasteele (Châtenay-Malabry), Pierre Vincent (Paris)

Registration fees: 50€ (acad), 250€ (non-acad) include meals and coffee breaks

Contact: biosensor-meeting@services.cnrs.fr

Information: http://www.cpps.u-psud.fr

Conception graphique : Ève RANVIER - Communication LCP - 2016 - Crédits photos : Université Paris-Sud

6th Biosensor Group Meeting, Orsay, 12-13 Mai 2016

Faculté des Sciences d'Orsay, Bâtiment des Colloques (Bat 338)

Thursday 12 May

14h - Session Mechanical Sensors, Cell Migration and Polarisation

Plenary 40+5 :

- **Carsten Grashoff (MPI Biochemistry, Munich)** How cells feel tissue stiffness - Second-generation tension sensors to quantify piconewton forces in cells.

Communications 20+5 :

- **Nicolas Borghi (IJM, Paris)** Mechanotransduction from cell surface to nucleus: roles of E-cadherin in catenin signalling .

- **Marc Tramier (IGDR, Rennes)**: Fluctuations of actomyosin-generated tensions on cadherin are similar between dividing and non-dividing epithelial cells in early Xenopus laevis embryon.

- **Adil Bakayan (BioEmergences, Gif sur Yvette)**: Visualizing and analyzing biological tension forces and their distribution

16h30 - Commercial Presentation

- **Hélène Delobel (Nikon, Champigny sur Marne)**: Innovative microscopy solutions for live cell imaging

16h45 Pause/Posters

17h30 - Session Analytical Imaging for Biosensing

Communications 20+5 :

- **Robert Pansu (ENS Cachan)** From lifetimes to concentrations in a FLIM image : the case of a precipitation reaction.

- **Vincent Gielen (LNBAA, KU Leuven)** Superresolution fluorescence microscopy of biomolecular interactions using photochromic biosensors and complementation.

- **Marcelina Cardoso dos Santos (IEF, Orsay)** : Time-Gated Terbium to Quantum Dots FRET Imaging for Intra- and Extracellular Biosensing.

19h30 - Conference Dinner

Interactive dinner with Monsieur Jacques (feat.)
Organized at Proto204 Creative Campus http://proto204.co

Friday 13 May

9h15 - Session Biosensors, Chemistry & Probes

Plenary 40+5 :

Ranieri Bizzarri (NEST, Nanoscience Institute, Pisa) New Optical probes and Imaging Strategy for Biology and Biomedicine.

Communications 20+5 :

- **Francisco Fueyo-González (Instituto de Quimica Medica, CSIC, Madrid)** Novel quinolimide-based solvatochromic and tunable fluorophores : development of CDK5 probes.

- **Marion Peyressatre (IBMM, Univ. Montpellier) :** Development of a fluorescent peptide biosensor for probing CDK5 activity and monitoring the efficacy of new therapeutics in glioblastoma.

11h00 - 11h30 Pause

- **Arnaud Gautier (UMR 8640, ENS, Paris)** : A genetically encodable fluorogen-based reporter for advanced biomolecular imaging.

- **Juan Antonio Gonzalez Vera (IBMM, Univ. Montpellier)** : Lanthanide-based peptide biosensor to monitor CDK4/cyclin D kinase activity.

12h30-14h30 - Buffet/Posters

14h30 - Biosensors for Cell Signaling : applications and recent developments

Communications 20+5 :

- **Ibrahim Bedioune (UMR-S 1180 INSERM, Chatenay)** Regulation of cytoplasmic and nuclear PKA activity by beta 1 and beta 2-adrenergic receptors in cardiac myocytes.

- **Dahdjim Betolngar (IBPS, UPMC, Paris)** NMDA transiently increases PDE1 activity in various brain regions

- **Giulia Bertolin (IGDR, Univ. Rennes):** FRET biosensor reveals the spatiotemporal activation and the functions of Aurora A in living cells.

- **Benjamin Cappe (IRC, Ghent University)** Spatio-temporal aspects and function of MLKL at plasma membrane during necroptosis.

- **Alexis De Angeli (I2BC, CNRS Gif-sur-Yvette) :** Sensing anion concentrations in Arabidopsis cells

Posters

- Florian Sizaire (IGDR, Univ. Rennes) Kinase activity biosensors: understanding the molecular mechanisms of activation of Aurora A and its interactome by FRET-FLIM

- **Nathalie Bouquier (IGF, CNRS Montpellier)** Activatable Cell-Penetrating Peptides (ACPPs) as biosensors of neuronal Matrix Metalloproteinase-9 activity.

- **Cédric Yapo (IBPS, UPMC Paris)** Adaptative responses to Dopamine signaling in the striatum of ageing and parkinsonian mice.

- **François Sipieter (IRC, Ghent University)** Spatio-temporal dynamic of MAPK/ERK during necroptosis.

- **Liang Zhang (INSERM, Chatenay Malabry)**, Role of PDE1 in controlling intracellular cAMP concentration in rat aortic smooth muscle cells and adult rat ventricular myocytes.

- **Claire Beauvineau (Institut Curie, Orsay)** A new fluorescent probe for identification of new ligands of G4- quadruplexes

- **Dorota Kostrz (LPN, Marcoussis)** Engineering FRET pairs to study the dynamics of protein conformational changes with a frequency-domain perturbative technique involving temperature oscillations

- **M. Pellerano (Institut Curie, Orsay)** Vinyltriphenylamines as fluorescent probes in biological applications

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Thursday 12 May Afternoon (1rst Part)

A. Session Mechanical sensors, cell migration and polarisation

How cells feel tissue stiffness

Second-generation tension sensors to quantify piconewton forces in cells"

Carsten Grashoff

Max Planck Institute of Biochemistry

Group of Molecular Mechanotransduction, Martinsried, Germany

Abstract: The ability of cells to adhere and simultaneously sense differences in tissue stiffness is inherent to all cells and crucial for organ development and function. Yet, the molecular mechanisms by which adherent cells sense extracellular matrix compliance have remained unknown because techniques to measure mechanical forces across intracellular adhesion structures were not available. We therefore developed two novel single-molecule–calibrated biosensors that allow the analysis of a previously inaccessible but physiologically highly relevant force regime. Our new probes are sensitive to 6-8 pN and 9-11 pN, they are characterized by an ultrafast folding/unfolding transition and a sharp force-response threshold.

By applying these biosensors to the cell adhesion protein talin, we demonstrate that this central integrin activator establishes mechanical linkages that bear forces between 7–10 pN and are regulated by f-actin and vinculin association. We find that the integrin-talin-actin linkage is indispensable for extracellular rigidity sensing and, surprisingly, talin isoform-specific.

Thus, we have developed additional biosensors to analyse mechanical forces in cells and our experiments identify talin as an essential regulator of extracellular rigidity sensing. As tissue stiffness changes during development or with the onset of disease states, it will be important to investigate the role of the individual talin-isoforms during these processes in more detail.

Mechanotransduction form cell surface to nucleus:
roles of E-cadherin in catenin signalling

Nicolas Borghi, Institut Jacques Monod, CNRS - Université Paris Diderot
Bât. Buffon - 15, rue Hélène Brion , 75205 Paris Cedex 13 - France

In multicellular organisms, cells generate and undergo mechanical forces that propagate through tissues. These forces may shape cells, tissues and organs, and also regulate genetic programs. We seek to elucidate the intimate mechanisms of the macromolecular complexes that transmit and transduce these mechanical cues within and between cells, and the cell functions affected by these cues.

We are currently interested in the intercellular adhesion complex comprising E-cadherins and catenins in epithelial cells. E-cadherins are transmembrane proteins that form intercellular bonds between adjacent cells and recruit cytoskeletal linkers catenins in the cytoplasm. Remarkably, catenins are also regulators of gene transcription when in the nucleus, and this activity may be triggered by biochemical as well as mechanical cues during major morphogenetic processes and disease. The roles of E-cadherins in the mechanical induction of catenin-dependent genes is, however, poorly understood.

I will present our recent advances in addressing this question, using genetically encoded biosensors and fluorescence microscopy.

Fluctuations of actomyosin-generated tensions on cadherin are similar between dividing and non-dividing epithelial cells in early Xenopus laevis embryo

Gaëtan Herbomel[1,2,3], Guillaume Hatte[1,2], Julien Roul[1,2,4], Sergi Padilla-Parra[1,2,5], Jean-Pierre Tassan[1,2], Marc Tramier[1,2].

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[3] Present adress: Section on Biological Chemistry, Department of Health and Human Services, NIDCR, National Institutes of Health, Bethesda, MD 20892, USA

[4] Present adress: LAAS-CNRS, Toulouse, France

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Organization and maintenance of tissues are regulated by mechanical forces inside and between cells, but only few quantitative data are available in-vivo. Following spatiotemporal variation of tension at cell-cell junction during xenopus embryo development is a real challenge to understand the role of mechanical forces in formation and homeostasis of tissues. During the last few years, new FRET biosensors have been developed that allow to directly measure mechanichal forces in living cells, using a tension sensor module inserted between two fluorescent proteins (Grashoff, et al., Nature, 2010). We focused on the Cadherin FRET biosensor developed by Nicolas Borghi (Borghi, et al., PNAS, 2012). Cadherin is localized at the apical pole of the cell and make a direct link between neighboring cells and the actin cytoskeleton via protein partner such α and β Catenin, making it the best candidate to study mechanical tension at the cell-cell junction. Using the fastFLIM prototype developed in the lab with Cadherin tension sensor, we are able to measure mechanical forces, with a picoNewton sensitivity, at cell-cell junctions in developing xenopus embryo. Our results suggest that at the end of blastula stage, fluctuations of actomyosin-generated tensions on cadherin are similar between dividing and non-dividing epithelial cells in early Xenopus laevis embryo with a mean tension of approximately 3pN.

6th Biosensor Meeting (Orsay)

Title: Visualizing and analyzing biological tension forces and their dynamics

Author list: Bakayan Adil, Dumont Julien, Peyriéras Nadine

The behavior of cells, individually or collectively, represents an elementary determinant for the proper development, organization and function of organisms. It is established that its mechanical properties play a major role in cell behavior and function: e.g. division, migration and differentiation. The mechanical architecture of the cell relies on the cytoskeleton and its constituents, the cytoskeleton signaling pathways interactions, and the integration of biomechanical cues and genetic regulation. In this context, biomechanical signaling and biochemical signals form the basis for cellular communication and the emergence of higher-level organization. The development of different readout modes to follow and quantify these forces is of growing interest. So far, the quantification of biomechanical forces in vivo is a measure issue. Tensor analysis studies allow determination of deformation rates and vectors but not forces. AFM techniques probe forces and allow an interpretation in the context of viscoelastic models that are certainly rough approximation of the biological tissues properties. In addition, laser ablations allow invasive investigation of the mechanics of the cytoskeleton and stress at cell and tissue level, respectively. Alternatively, analysis of membrane shape helps estimate stress from the boundary of cell membranes although not yet established in 3D+time. In fact, the first methodological approach that tackled the *in vivo* mechanical force measurements in 3D was the fluorescent lipid micro-droplets. Moreover, a growing number of excellent biosensors based on fluorescence as readout mode have the potential to visualize and analyze the dynamics mechanotransduction at the level of specific molecular assemblies. Multiplexing these different paradigms to integrate a global and precise representation of force dynamics is a great challenge.

Innovative microscopy solutions for live cell imaging

Hélène Delobel
Product Manager, Nikon France S.A.S, Champigny sur Marne

To address understanding of living cells, microscopy company like Nikon develop innovative imaging solutions.

This talk will introduce two different light patterned approaches designed to help researchers in their living cell studies.

The first one is based on Nikon technologies, the Ti-Lapp system and its modular NIS software. This versatile multimodal illumination system offers a simple way to achieve optogenetic experiments on living cells.

In the second part, the new Primo technology from Alvéole, the first multi-protein printing platform will be presented.

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Thursday 12 May Afternoon (2nd Part)

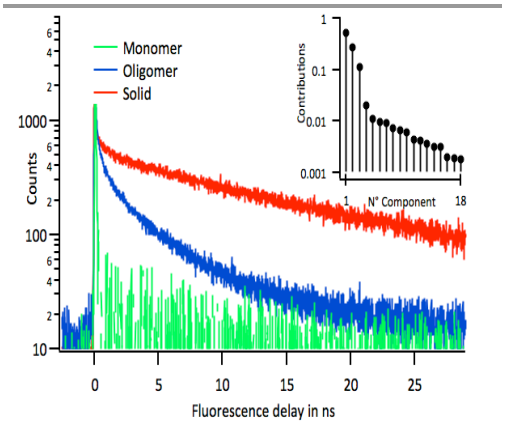
B - Session Analytical imaging for biosensing

Analyse par FLIM d'une réaction de précipitation.[1]

Vu Long Tran, Valérie Génot, Jean-Frédéric Audibert, Yury Prokazov,[±] Evgeniy Turbin,[±] Werner Zuschratter,[±] Hyeong-Ju Kim,[°] Jaehun Jung,[°] Soo Young Park[°] et Robert B. Pansu*

L'imagerie FLIM par comptage de photon produit des déclins de fluorescence en chaque pixel d'une image (Jusqu'à un million dans notre cas)[2]. L'analyse d'un déclin par déconvolution est déjà une tâche délicate[3]. L'analyse d'une image FLIM peut prendre un temps infini. C'est pourquoi des méthodes alternatives ont été proposées comme la technique Phasor[4] où une représentation graphique des résultats d'une transformation de fourrier des données permet d'estimer si un pixel est mono-, bi- ou multiexponentiel. Nous proposons une approche sans déconvolution, sans hypothèse, robuste et rapide pour analyser les images FLIM.

Nous avons montré qu'une analyse en composantes principales[5] permet de répondre à trois questions 1- Combien d'espèces contribuent au déclin de fluorescence ? 2- Quels sont leurs déclins ? 3- Où sont-elles localisées dans l'image ? La méthode sera illustrée par un enregistrement par vidéo-FLIM d'une réaction en microfluidique qui allie imagerie et cinétique.



L'analyse par PCA et les déclins de 3 espèces , obtenus dans des zones particulières du dispositif.

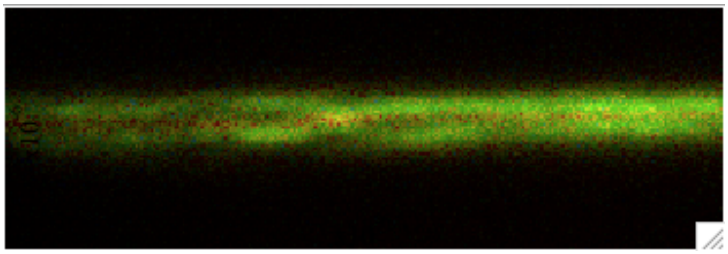
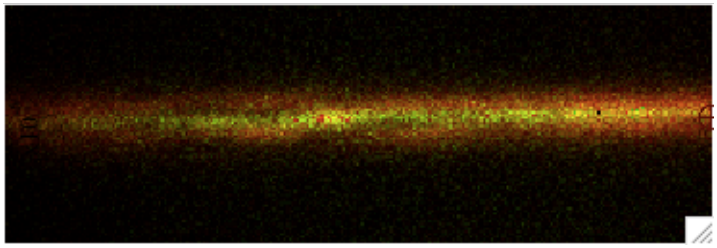


Image FLIM

Co-localisation



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6th Biosensor Meeting

Abstract

Superresolution fluorescence microscopy of biomolecular interactions using photochromic biosensors and complementation

Vincent Gielen[1], Sam Duwé[1], Wim Vandenberg[1], Johan Hofkens[2], and Peter Dedecker[1]

[1] Laboratory for Nanoscale Biological Architecture and Activity, Department of Chemistry, KU Leuven

[2] Molecular Imaging and Photonics, Department of Chemistry, KU Leuven

In the last two decades fluorescence microscopy has become an indispensable tool for the real-time study of biomolecules and biochemical pathways in living cells, tissues, and whole animals. The discovery and development of fluorescent proteins (FPs), and recent advances in diffraction-unlimited far-field optical microscopy have revolutionized modern-day biological research even further.[1] However, the labels still remain a severely limiting factor, especially for super-resolution microscopy where the demands are considerably high.[2] Many of these techniques require the use of 'photophysically smart labels' such as reversibly photoswitchable fluorophores.[3] Engineered fluorescent proteins have provided some of the best such labels, and as a result are frequently used for advanced fluorescence imaging. However, in the majority of cases these uses have remained limited to visualizing the locations of the fluorophores, rather than being useful as biosensors.

In this presentation I will describe my work, where I combine these interesting implementations of FP technology in order to create two types of photoswitchable biosensors: on the one hand highlightable calcium indicators, on the other hand bimolecular fluorescence complementation fragments, based on fluorescent proteins. These hybrid tools would enable interaction measurements and sensing in living biological systems, on a nanoscale.

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Time-Gated Terbium to Quantum Dots FRET Imaging for Intra- and Extracellular Biosensing

M. Cardoso Dos Santos *, H.S. Afsari **, S. Lindén *, T. Chen **, X. Qiu *, P.M.P. van Bergen en Henegouwen ***, T.L. Jennings ****, K. Susumu *****, I.L. Medintz ******, N. Hildebrandt *, L.W. Miller **

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Time-gated FRET using the unique material combination of long-lifetime terbium complexes (Tb) and semiconductor quantum dots (QDs) provides many advantages for highly sensitive and multiplexed biosensing. Although time-gated detection can efficiently suppress sample autofluorescence and background fluorescence from directly excited FRET acceptors, Tb-to-QD FRET has been rarely exploited for biomolecular imaging [1,2]. In this study, we highlight Tb-to-QD time-gated FRET biosensing that can be applied for intra and extracellular imaging (Fig.1). Immunostaining of different epitopes of the epidermal growth factor receptor (EGFR) with Tb and QD conjugated antibodies and nanobodies allowed for efficient Tb-to-QD FRET on A431 cell membranes. The broad usability of Tb-to-QD FRET was further demonstrated by intracellular multicolor Tb-to-QD FRET or Tb-to-QD-to-dye FRET using microinjection as well as cell penetrating peptide mediated endocytosis with HeLa cells. Multiplexed biosensing at very low concentrations (~nM), and the quick and sensitive detection void of FRET acceptor background fluorescence are highly important advantages for advanced live cell imaging of biomolecular interactions.

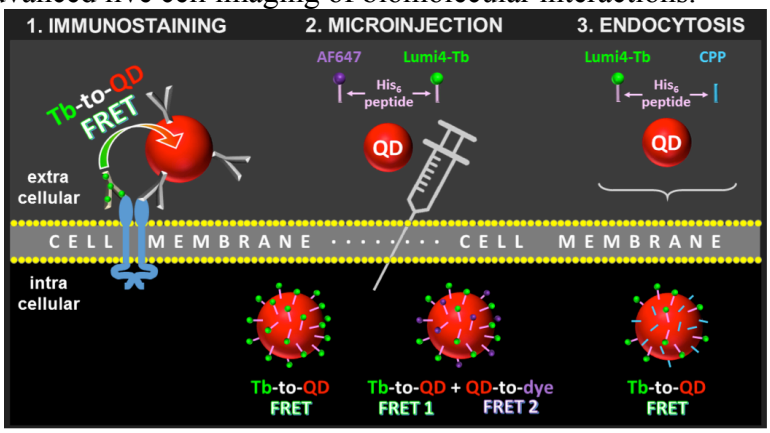

Fig. 1. Schematic presentation of the FRET imaging approaches: 1. Extracellular FRET between Tb and QD functionalized antibodies recognizing cell membrane receptors. 2. Intracellular (cytosol) FRET from Tb-to-QD and FRET relays from Tb-to-QD-to-dye using microinjected self-assemblies. 3. Intracellular (endosomes/lysosomes) FRET from Tb-to-QD using CPP-mediated endocytosis.

[1] O. Faklaris, M. Cottet, A. Falco, B. Villier, M. Laget, J.M. Zwier, E. Trinquet, B. Mouillac, J.P. Pin, T. Durroux, "Multicolor time-resolved Forster resonance energy transfer microscopy reveals the impact of GPCR oligomerization on internalization processes", FASEB J., 29., 2235-2246, (2015).

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Faculté des Sciences d'Orsay, Bâtiment des Colloques (Bat 338)

Friday 13 May Morning

C - Session "Biosensors, Chemistry & Probes"

New Optical probes and Imaging Strategy for Biology and Biomedicine

Ranieri Bizzarri
NEST, Istituto Nanoscienze – CNR, piazza San Silvestro 12, 56127 Pisa

The arsenal of fluorescent probes tailored to functional imaging of cells is rapidly growing and benefits from recent developments in imaging strategies. Fluorescent sensors of polarity and viscosity at nanoscale are particularly interesting for high-resolution microscopy imaging and diagnostics of living cells as these physicochemical properties modulate many cellular processes [1-3]. Ideally, polarity/viscosity probes should fulfill these requirements: a) optical responses (intensity, wavelength-shift, lifetime) predictably related to the environmental polarity or viscosity changes; b) strong brightness for high-sensitivity detection; c) easy conjugation to biomolecules. Conventional probes sense local polarity as expressed by orientation polarizability, which depends in a complicated way on both local static dielectric constant ε and refractive index [4]. Here, I shall review our activity in the field of environmental fluorescent probes for intracellular use. In particular, we shall focus on a visible-absorbing/emitting fluorescent probe, structurally similar to the GFP chromophore, which efficiently reports on ε with good accuracy both in vitro and in living cells [1]. As for living cells, using confocal microscopy we obtained spatially resolved ε maps for many subcellular compartments, such as endoplasmic reticulum, nuclear envelope, and plasma membrane. From a photophysical point of view, we demonstrated that this probe behaves also as a molecular rotor, allowing for the measurement of the fluidity of local environments through lifetime. Accordingly, we determined maps of local membrane fluidity in living cells at physiological and non-physiological conditions by Fluorescence Lifetime Imaging (FLIM) both in conventional and "phasor" mode [5]. Additionally, we shall discuss on simpler viscosity-sensitive probes that allow for monitoring local viscosity in plasma membrane, lysosomes, mitochondria [6], and chromatin [7]. Finally, I shall discuss the use of reversibly photoswitchable (photochromic) fluorescent proteins, which have become an invaluable tool for the optical labeling and tracking of living cells, organelles and intracellular molecules in a spatio-temporal manner [8]. We recently showed that synthetic chromophores isolated from *Aequorea Victoria* (AFPs) present a reversible *cis-trans* photoisomerization mechanism similar to that reported for photochromic FPs from *Anthozoa* or other organisms [9]. Owing to the relevance of AFPs for cell biology, a photochromic "toolbox" constituted by several AFPs is highly desirable. The known photochromic AFP mutants, however, are far from being optimized, since they need to absorb ~10^6 photons to carry out the on-off photoconversion [10], and their photochromic behaviour was often reported to be accompanied by other undesirable effects (e.g. irreversible photoconversion [11]). Here I shall describe new photochromic AFPs whose reversible photoswitching occurs between the native bright and a dark state at low illumination power, owing to a very efficient *cis-trans* photoisomerization (~10^3 photons for switching) [12, 13]. Most remarkably, the optical bistability of these AFPs derives from a single mutation in the primary sequence of otherwise photochemically stable popular AFP variants. The significance of these mutants for high-resolution cell imaging will be shown by means of super-resolution imaging schemes such as Stochastic Optical Fluorescence Imaging (SOFI) and PhotoActivation Localization Microscopy (PALM) applied to unveil the intracellular organization of key proteins at nanoscale.

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NOVEL QUINOLIMIDE-BASED SOLVATOCHROMIC AND TUNABLE FLUOROPHORES: DEVELOPMENT OF CDK5 PROBES

Francisco J. Fueyo-González,[a] Juan A. González-Vera,[a,b] Marion Peyressatre,[b] May C. Morris[b] and Rosario Herranz[a]

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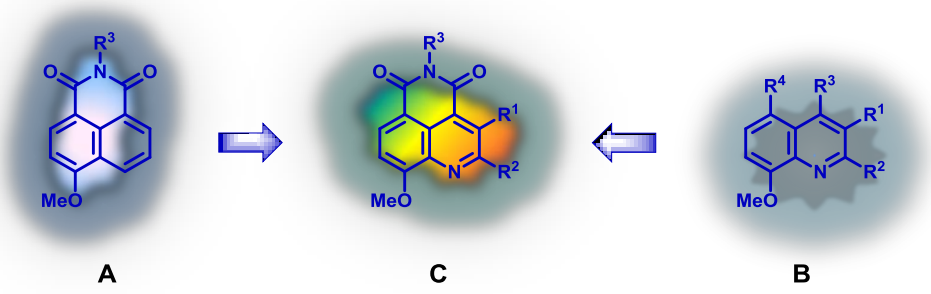
Solvatochromic fluorophores have been used in the design of biosensors with applications ranging from the study of protein structural dynamics to the detection of protein-binding interactions. 1,8-Naphthalimide-based biosensors have recently attracted considerable interest in this field, due to their favorable photophysical properties, such as strong absorption and emission, large Stokes shifts, and photostability. However, they have the drawback of their limited water solubility, necessary for protein detection studies. Taking into account that introduction of a protonable nitrogen atom into the 1,8-naphthalimide scaffold (**A**) could increase water solubility and red-shift the fluorescence emission, along with the prevalence of the quinoline core (**B**) in diverse fluorescence sensors, we have synthesized and explored the fluorescence properties of chimeric naphtalimide-quinoline fluorophores (**C**). In this communication we report the design, synthesis and photophysical properties of novel 4,5-quinolimide-based fluorophores, as well as, their application to CDK5 peptide probes.



Acknowledgment: The work was supported by the Spanish Ministerio de Economía y Competitividad grant SAF2012-32209 and the CSIC grant 2012280E096. J.A.G.V. is supported by a Marie-Curie fellowship EC-FP7 Framework.

Development of a fluorescent peptide biosensor for probing CDK5 activity and monitoring the efficacy of new therapeutics in glioblastoma

Marion Peyressatre & May C. Morris

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CDK5 is a neurospecific kinase activated in the central nervous system through association with its regulatory partners p35 and p39 which is implicated in neuronal functions such as synaptic transmission, axonal guidance and migration (1,2). Calpain-mediated cleavage of p35 to p25 and subsequent hyperactivation of CDK5 are associated with neuronal death in post-mitotic cells and several neurodegenerative diseases (3). More recent studies suggest that CDK5 expression and hyperactivation are involved in glioblastoma during cell invasion and CDK5 expression has been reported to be correlated with the pathological grade of gliomas (4,5,6).

There are currently no tools available to monitor CDK5/p25 activity in its native environment in a sensitive and continuous fashion. We have developed a fluorescent peptide biosensor named CDKACT5, which selectively reports on recombinant CDK5/p25 and on endogenous CDK5 activity in cell extracts upon stimulation or inhibition of this kinase in a dynamic and reversible fashion. Moreover, introduction of CDKACT5 into cultured neuronal cells reports on sensitive and rapid changes in CDK5/p25 activity by fluorescence imaging following activation or inhibition (Peyressatre et al., in preparation).

More recently, we have identified novel non-ATP competitive modulators of CDK5 in a high throughput screen of 3 small molecule libraries thanks to a biosensor that reports on conformational modulators of this kinase. The efficacy of the hit compounds, expected to behave as inhibitors of allosteric inhibitors of CDK5/p25, have been characterized thanks to CDKACT5 *in vitro* and living cells by fluorescence imaging.

These fluorescent biosensors constitute sensitive and potent tools for addressing issues related to the dynamics of CDK5 activation in neuronal cells in physiological and in pathological conditions, offering new opportunities to monitor and quantify its hyperactivation in neurodegenerative diseases and cancer.

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A genetically encodable fluorogen-based reporter for advanced biomolecular imaging

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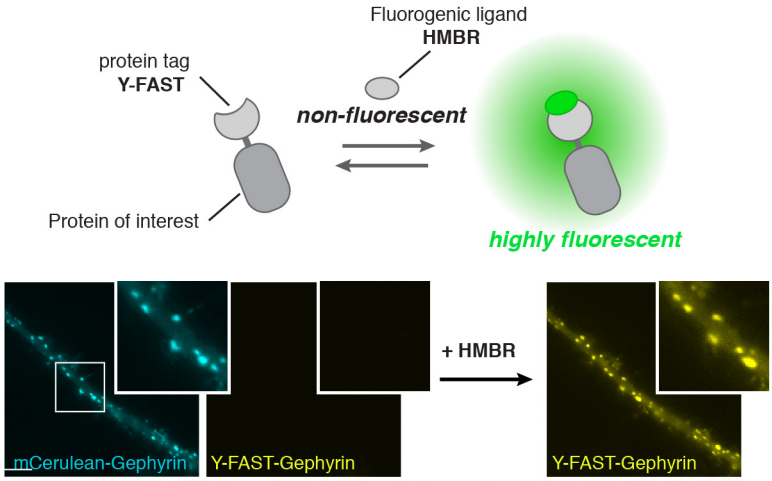
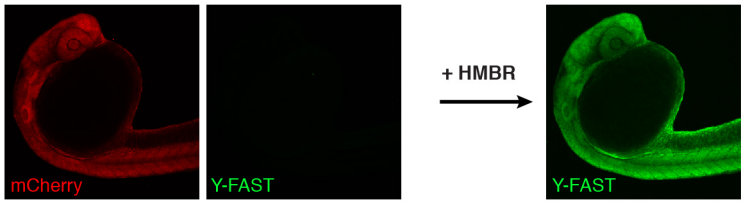
Biological imaging is essential for revealing the inner workings of living systems. Among the numerous imaging modalities, light microscopy has revolutionized biological research. Microscopes that enable fluorescence imaging in live cells and animals have been indispensable in our current understanding of biological processes. In addition to advances in optics and detectors, imaging has benefited from the development of molecular tools to observe biomolecules in action. During this presentation, I will present the development of a tunable protein tag dubbed Y-FAST (Yellow Fluorescence-Activating and absorption-Shifting Tag). Y-FAST fluoresces instantaneously upon binding a cell-permeant and non-toxic synthetic fluorogenic dye (so-called fluorogen). A unique fluorogen activation mechanism based on two spectroscopic changes — increase of fluorescence quantum yield and absorption red shift — ensures high selectivity and high contrast. Y-FAST was engineered from the 14-kDa photoactive yellow protein (PYP) by directed evolution. Y-FAST compares favorably to common GFP-like fluorescent proteins in terms of brightness and photostability and functions in diverse organelles, cells and organisms. Y-FAST distinguishes itself from other tagging systems because fluorogen binding is rapid, highly dynamic and fully reversible. This latter feature makes Y-FAST a genetically encodable fluorescence on/off switch holding great potentials for multiplexing imaging, super-resolution microscopy and biosensing.



Selective labeling of Y-FAST-Gephyrin enables the visualization of inhibitory synapses in dissociated spinal cord neurons



Selective labeling of cytoplasmic Y-FAST in zebrafish embryo

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Lanthanide-based peptide biosensor to monitor CDK4/cyclin D kinase activity

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Cyclin-dependent kinases (CDK/cyclins) play a central role in coordinating cell cycle progression, and in sustaining proliferation of cancer cells, thereby constituting established cancer biomarkers and attractive pharmacological targets. In particular, CDK4/cyclin D is frequently altered in lung cancer, melanoma and lymphoma.[1]

Fluorescent biosensors constitute potent tools for probing biomolecules in complex biological samples, living cells and organisms in real-time, in a reversible and non-invasive fashion. Lanthanide ions represent attractive complements to the more frequently used organic fluorophores, as these species exhibit long-lived (millisecond) luminescence lifetimes, large Stokes shifts, and sharp emission peaks.[2-3] The long-lived luminescence of lanthanides, being significantly longer than the nanosecond lifetimes of organic fluorophores, provides greatly increased sensitivity by elimination of the background natural fluorescence.

In order to develop tools to monitor CDK4/cyclin D kinase activity, which feature high signal to noise ratio, we have designed and engineered a lanthanide-based peptide biosensor that specifically reports on CDK4 phosphorylation through sensitive changes in luminescence intensity. The designed biosensor includes a kinase recognition motif, a phosphoamino acid binding domain (PAABD), and a sensing unit, consisting of one Trp residue localized at PAABD acting as lanthanide sensitizer, and the DOTA-Tb^{3+} complex, close to the phosphorylatable residue. This modular system was first validated, by comparing the luminescence intensity of synthetic phosphorylated and unphosphorylated peptides, showing high luminescence enhancements (3-5 fold). Afterwards, the biosensor was validated *in vitro* using melanoma cell extracts (A375 cell line).

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6th Biosensor Group Meeting, Orsay, 12-13 Mai 2016

Faculté des Sciences d'Orsay, Bâtiment des Colloques (Bat 338)

Friday 13 May Afternoon

D - Session Biosensors for cell signaling : applications and recent developments

DIFFERENTIAL REGULATION OF CYTOPLASMIC AND NUCLEAR PKA ACTIVITIES BY β_1- AND β_2-ADRENOCEPTORS IN ADULT CARDIAC MYOCYTES

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Functional differences between β_1-adrenoceptors (β_1-ARs) and β_2-ARs in the regulation of cardiac excitation-contraction coupling have been widely studied. Much less is known regarding differences between β_1- and β_2-ARs in the regulation of gene expression and the mechanisms involved. Here, we studied the mechanisms regulating nuclear *versus* bulk cytoplasmic PKA activation by β_1- and β_2-ARs and the functional consequences on gene expression.

PKA activity was measured using targeted FRET-based A-kinase activity reporters. For specific β_1- or β_2-AR stimulation, isoprenaline (Iso) was used in combination with the β_2-AR antagonist ICI118551 or with the β_1-AR antagonist CGP20712A, respectively. At all Iso concentrations tested, β_1-AR stimulation was more efficient than β_2-AR stimulation to increase cytoplasmic and nuclear PKA activities. For a similar activation of cytoplasmic PKA, nuclear PKA activity was 3-fold higher with β_1-AR than with β_2-AR stimulation. Inhibition of G_i protein, caveolae disruption, and inhibition of GRK2-mediated desensitization potentiated β_2-AR-induced cytoplasmic but not nuclear PKA activity. PDE4 inhibition strongly potentiated cytoplasmic and nuclear PKA responses to both β_1- and β_2-AR stimulation. In contrast, PDE3 inhibition had no significant effect on β_1-AR induced PKA activation in both compartments, while it increased cytoplasmic but not nuclear PKA activity upon β_2-AR stimulation. Downregulation of mAKAP, a nuclear envelope associated scaffold protein, decreased β_1-AR stimulation of nuclear PKA activity. Consistently, β_1-ARs but not β_2-ARs were able to induce the expression of the PKA-regulated pro-apoptotic gene, ICER. These results show that i) β_1- and β_2-ARs differentially regulate cytoplasmic versus nuclear PKA activities, ii) nuclear PKA activation can be dissociated from bulk cytoplasmic PKA activity upon β_2-AR stimulation; and iii) PDE4 and mAKAP are critical components of nuclear PKA signalling.

NMDA transiently increases PDE1 activity in various brain regions.

Authors

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Abstract

Type 1 phosphodiesterases (PDE1) are characterized by their activation by Ca2+/calmodulin and exhibit a dual selectivity for both cAMP and cGMP. The three different isoforms of PDE1 are expressed in various brain regions but their impact on cyclic nucleotide control under different conditions are poorly understood. Here, we used a new selective PDE1 inhibitor AF64196 to analyze the functional role of PDE1 in different neuronal types.

A novel biosensor for cGMP was developed, comprising a cGMP binding domain from the regulatory domain of type I cGMP-dependent protein kinase, fused between a mCerulean3 and YPET FRET pair. This biosensor exhibited a large ratio change and high selectivity for cGMP against cAMP. This biosensor, or the cAMP biosensor Epac-S^{H150}, was expressed in brain slices from 8-12 days old mice, imaged with wide-field fluorescence microscopy, and the ratio was monitored to analyze the changes in intracellular cGMP or cAMP. Brain slices from the prefrontal cortex, the hippocampus and the dorsal striatum were examined.

In order to reveal a calcium-activated PDE1 activity, cAMP or cGMP production was first stimulated using forskolin, a direct activator of adenylyl cyclase, or with DEANO, a NO donor, respectively. After reaching a steady-state cAMP or cGMP level, NMDA was released from a caged derivative (100 µM NPEC-NMDA) by a flash of UV light: this produced transient decreases in cAMP or cGMP, which was dose-dependently blocked by AF64196 and therefore attributable to a transient increase in PDE1 activity.

NMDA-triggered PDE1 activation was observed in all three brain regions examined with this protocol, but there were qualitative and quantitative differences between the regions. Overall, the results suggest that PDE1 inhibitors may have potential therapeutic effects through the regulation of NMDA-mediated synaptic plasticity.

A FRET biosensor reveals the spatiotemporal activation and the functions of Aurora A in living cells.

Giulia Bertolin[1,2], Florian Sizaire[1,2], Gaëtan Herbomel[1,2] David Reboutier[1,2], Claude Prigent[1,2] and Marc Tramier[1,2]

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Overexpression of *AURKA* is a major hallmark of epithelial cancers. This gene encodes the multifunctional serine/threonine kinase Aurora A, which regulates the maturation of centrosomes, the assembly of the mitotic spindle and the timing of the mitotic entry when activated in the G2/M phase of the cell cycle. In this phase, autophosphorylation of Aurora A on Thr288 modifies the conformation of the catalytic loop of Aurora A, and it allows the kinase to switch from an auto-inhibited state to an activated one. For next-generation drug design, tracking the autophosphorylation on Thr288 and the conformational changes of Aurora A in living cells is mandatory to assess the multiple roles of this kinase. We described here a novel Förster's Resonance Energy Transfer (FRET) biosensor, which detects the conformational changes of AURORA A following its activation by autophosphorylation on Thr288 *in vitro* and *in cellulo*. Two widely used inhibitors of the catalytic activity of Aurora A, MLN8327 and MLN8054 prevented the conformational changes of the biosensor by blocking autophosphorylation on Thr288. We demonstrated that the biosensor functionally replacs the endogenous Aurora A in living cells, and for the first time it allowed to follow the activation of the kinase throughout the cell cycle. With this tool, we discovered that Aurora A activates during the G1 phase to regulate the stability of the microtubule network in cooperation with the microtubule-associated protein TPX2 and the centrosomal protein CEP192, previously shown to interact with Aurora A at mitosis. These results provide evidence that the Aurora A biosensor is a powerful tool to identify new regulatory pathways controlling Aurora A activation. The development of novel Aurora A inhibitors targeting specific conformational changes of the kinase may represent a promising strategy in anti-cancer therapies.

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Abstract for Biosensor Meeting - 2016

Title: Spatio-temporal aspects and function of MLKL at plasma membrane during necroptosis

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Abstract (max 500 words):
Do you know that over a million of cells are dying each day in your body and are replaced by other ones? However, cell death can take different forms. For a long time, apoptosis was considered as the only form of regulated cell death, and necrosis was considered as accidental. Over the 2 past decades, necrosis was shown to be driven by different pathways. Nowadays, necroptosis, a necrotic regulated and caspase-independent cell death is intensively studied. Moreover, an increasing number of studies underline an important role of regulated necrosis in diseases (e.g. Cröhn disease, ischemia-reperfusion injuries…).

The molecular events governing necroptosis have been extensively studied during the past years, and the important role of receptor interacting protein kinases (RIPK) 1 and 3 has been established. In 2012, the pseudo-kinase Mixed-Lineage Kinase Like (MLKL) was shown to be downstream of RIPK3 and the crucial executioner of necroptosis. Upon RIPK3 mediated MLKL phosphoryltion, MLKL oligomerizes and is believed to relocalizes to the plasma membrane. An increasing number of reports highlighted the importance of MLKL oligomerization for its function. However, MLKL function is still controverted in the literature, but our recent data, in accordance with some recent studies, advocate for a pore-forming capacity of MLKL.

The sequence of major molecular events during necroptosis has become clearer, and most of the knowledge about this pathway came from biochemical approaches that gave a snap-shot view of what is happening in a cell population. However, processes are highly dynamic and are not synchronized in a population. Up-to-now, MLKL dynamics has never being studied under TNF stimulation (i.e. localization, interaction with RIPK3, oligomerization, plasma membrane translocation). In order to tackle MLKL dynamics and determine its function in regulated necrosis, we developed and are validating a cell line that enables us to monitor MLKL behavior upon TNF-induced necroptosis and visualized MLKL in realtime at the single living cells level. Using advanced fluorescence imaging techniques such as TIRF (Total Internal Reflexion Fluorescence) we witness for the very first time in TNF-induced necroptosis, MLKL punctuate structure formation few minutes before plasma membrane rupture.

Sensing anion concentrations in Arabidopsis cells

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In plants, as in all other organisms, ion transport plays an important role in several physiological processes. In the last decade several membrane proteins responsible of ion transport have been identified in the model plant Arabidopsis. This dramatically increased our understanding of the molecular basis of ion distribution within the cell and among organs. Anions play a crucial role in plant cells as they participate to membrane depolarization during signaling events, to nutrition and to abiotic stress tolerance. However, little information is available about the intracellular anionic concentrations and anion fluxes among plant cell compartments. To measure *in vivo* anion concentrations we use genetically encoded anion sensors able to sense monovalent anions in the cytosol independently from pH variations. We will report the *in vivo* characterization of the ClopHensor chloride sensor in Arabidopsis cells.

6th Biosensor Group Meeting, Orsay, 12-13 Mai 2016

Faculté des Sciences d'Orsay, Bâtiment des Colloques (Bat 338)

Posters

Kinase activity biosensors: understanding the molecular mechanisms of activation of Aurora A and its interactome by FRET-FLIM

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Overexpression of AURKA is a major hallmark of epithelial cancers. This gene encodes the multifunctional serine/threonine kinase AURKA, which is activated at metaphase and is required for cell cycle progression. Conventional techniques like Western Blot, Radiometry or Immunofluorescence have limitations when one wants to investigate dynamic spatiotemporal regulation of a kinase in living cells.

Our team has recently developed a FRET biosensor for Aurora A consisting of the whole kinase flanking by two fluorophores, GFP and mCherry. We showed that the change of conformation of Aurora A when activated by the phosphorylation of T288 will bring closer GFP and mCherry allowing FRET. Efficiency of FRET depends on proximity of the two fluorophores and this phenomenon leads to a decrease of donor fluorescence lifetime. By using FLIM to measure variations of FRET efficiency, we are able to follow activation of Aurora A through cell cycle in time and in space in a living cell expressing the biosensor at endogenous levels.

Firstly, we aim to refine our approach by developing a methodology based on multiplex FRET to follow simultaneously Aurora A activation and a coactivator interaction such as TPX2. In this case, we will use Aurora A biosensor for the activation and the co-activator labelled with a far-red fluorophore. By monitoring fluorescence lifetime of GFP and of mCherry we could associate activation or inactivation of Aurora A with direct interaction with its partner. It will provide a new strategy to investigate partners of Aurora A and to associate them to its multiple functions during the different phases of the cell cycle.

Secondly, we will use our biosensor to perform inhibitor screening test. This project would be based on ratiometric measurement of GFP and mCherry fluorescence intensities to record variations of FRET according to the inhibitor. By using an automated pipeline, we would be able to screen a large panel of inhibitory molecules. It will provide a new screening approach based on direct monitoring of kinase activation and inactivation.

Activatable Cell-Penetrating Peptides (ACPPs) as biosensors of neuronal Matrix Metalloproteinase-9 activity.

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Activatable Cell Penetrating Peptides (ACPPs) are biosensors comprised of a polycationic sequence connected to a neutralizing polyanion via a cleavable linker. Proteolysis of the linker by a specific protease affords dissociation of both domains and enables the polycationic CPP to enter into the cell via an endocytosis process. A cargo such as an imaging agent or a therapeutic molecule can be attached to the CPP polycation. We propose to develop an ACPP sensitive to Matrix Metalloproteinase-9 (MMP-9) activity. This protease has recently emerged as a major element of brain physiology and pathology as it plays a critical role in synaptic changes associated with learning and memory. Two versions of ACPPs were designed. First, recombinant peptides consisting of ACPPs boxed between Bioluminescence Resonance Energy Transfer (BRET) compatible entities (NanoLuciferase and Venus proteins) exhibit sensitivity to MMP9 protease activity. Second, synthetic ACPPs coupled to a red fluorophore are used to measure uptake of fluorescence in hippocampal neurons and will be tested for *in vivo* applications. Cleavage of both ACPPs biosensors by active secreted MMP-9 in response to enhanced neuronal activity will allow us to monitor enzyme activity by cell imaging. Thus, the use of ACPPs as molecular imaging probes is a promising new approach for the visualization of enzyme activity and targeting synaptic dysfunctions.

ADAPTATIVE RESPONSES TO DOPAMINE SIGNALING IN THE STRIATUM OF AGEING AND PARKINSONIAN MICE.

Authors

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Abstract

Dopamine (DA) is involved in the regulation of various functions (motor behaviors, reward, learning, etc.), and dysfunctions in DA signaling are associated with numerous neuropsychiatric diseases, including schizophrenia and Parkinson’s disease.

DA exerts its cellular effects mainly by modulating the cAMP/PKA pathway, which run is critical for the regulation of neuronal excitability or long-term plasticity for instance. In the main input nucleus of the Basal Ganglia, i.e. the striatum, DA modulates the activity of two resembling groups of GABAergic neurons, the striatonigral and striatopallidal Medium Spiny Neurons (MSNs). The striatonigral MSNs preferentially express D1 receptors, while the striatopallidal MSNs preferentially express D2 receptors. These two DA receptor subtypes are oppositely coupled to the cAMP/PKA pathway.

For years, our team has been using biosensor imaging to study the dynamics of the cAMP/ PKA pathway in the MSNs. Previous work in our lab notably showed that the striatonigral MSNs had a very specific ability to encode brief dopaminergic stimulations, such as those involved in goal-directed learning (Castro et al., 2013). Ongoing work aims at studying the changes that occur in the functional organization of this signaling pathway, in mice suffering from chronic affections of the dopaminergic system.I

Spatio-temporal dynamic of MAPK/ERK during necroptosis

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Abstract:
Necroptosis is defined as a caspase-independent programmed cell death and relies on a signaling pathway involving two serine-threonine kinases: Receptor-Interacting Protein Kinase 1 and 3 (**RIPK1** and **RIPK3**) and the pseudo-kinase Mixed-Lineage Kinase Like (**MLKL**). Activation of Extracellular signal-Regulated Kinases 1 and 2 (ERK1/2) was reported to be involved in different modes of programmed cell death. It is now accepted that the regulation of the duration, magnitude and subcellular compartmentalization of ERK1/2 activity by specific **spatio-temporal regulators** is interpreted by the cell towards **cell fate determination**. ERK1/2 inhibition delays TNFα-induced necroptosis in L929 cells in a dose dependent manner but did not block it, providing arguments for **a pro-necrotic function of ERK1/2**. In this context, a compartmentalized biphasic phosphorylation of ERK1/2 was observed. Our results indicate a RIPK1-dependent phosphorylation of ERK1/2. Owing to the importance of ERK1/2 spatio-temporal dynamics in determining cellular responses, we developed a new reporter of ERK2 localization named **ERK2-LOC**. We observed a transient translocation of ERK2 when necroptosis was triggered in L929 upon TNFα stimulation, followed by progressive ERK2 accumulation in the nucleus. ERK1/2 activities were monitored during necroptosis using a FRET-based kinase biosensor for ERK1/2 (ERK1/2-ACT). Using **ERK1/2-ACT**, a dedicated spatio-temporal signature of ERK1/2 activity was recorded during necroptosis. Finally, to correlate ERK1/2 activity code with necroptosis occurrence, we also engineered a first generation of FRET biosensors to report on both RIPK1 and RIPK3 activities during necroptosis.

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ROLE OF PDE1 IN CONTROLLING INTRACELLULAR cAMP CONCENTRATION IN RAT AORTIC SMOOTH MUSCLE CELLS AND ADULT RAT VENTRICULAR MYOCYTES

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Background: Recently, the cyclic nucleotide phosphodiesterase 1 (PDE1) family has been demonstrated to play important roles in cardiovascular system. Here, we took advantage of the development of a new selective PDE1 inhibitor, PF-04471141 (PF1, 1µM), and measured its effect on intracellular cAMP concentration ($[cAMP]_i$) in cultured rat aortic smooth muscle cells (RASMCs) and adult rat ventricular myocytes (ARVMs) under various conditions.
Methods: PDE activity was measured in the protein extracts of RASMCs and ARVMs 48h after plating. $[cAMP]_i$ was recorded by fluorescence microscopy in cultured RASMCs and ARVMs 48h after infection with a fluorescence resonance energy transfer (FRET)-based cAMP sensor.
Results: In the presence of Ca^{2+} and calmodulin (CaM), cAMP-PDE activity and cGMP-PDE activity were significantly increased by 20% and 65%, respectively in RASMCs, but only cGMP-PDE activity was significantly increased in ARVMs. PF1 significantly inhibited Ca^{2+}/CaM-activated PDE activity. In RASMCs, PF1 produced no effect on $[cAMP]_i$ under basal condition or after isoprenaline (Iso, 10 or 100 nM) stimulation. When Iso was applied in the presence of Angiotensin II (AngII, 400 nM), the $[cAMP]_i$ response to Iso pulse (15 s) returned to basal with a faster kinetic than with Iso alone, indicative of a higher hydrolysis of cAMP. This effect was partially blocked by PF1. In ARVMs, PF1 increased the amplitude of Iso response but had no effect on its kinetics. Unlike PF1, Ro-20-1724 (10 µM), a PDE4 inhibitor, produced a large increase in the $[cAMP]_i$ response to Iso, both in RASMCs and ARVMs. However, PF1 had no additional effect under these conditions.
Conclusions: PF1 exerted negligible effect on basal or Iso-stimulated $[cAMP]_i$ in RASMCs and ARVMs, except in the presence of AngII in RASMCs. This effect might be related to AngII-induced activation of PDE1 activity through increase in Ca^{2+} concentration [1].

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A new fluorescent probe for identification of new ligands of G4-quadruplexes

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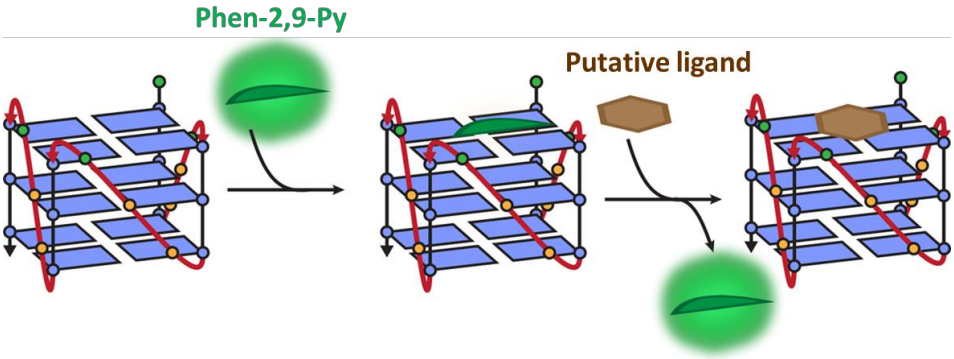
G4-quadruplexes are suspected to be involved in the regulation of various gene expressions as they interfere with events related to the transfer and maintenance of genetic information (replication, transcription, telomeres maintenance, translation). Small molecules can be very efficient tools to decipher their biological roles.[1]

The identification of new G4-ligands is still of high interest and requires a robust and easy assay to evaluate quickly compounds on a panel of G4 sequences. In this context, our laboratory developed the G4-FID-assay (Fluorescent Intercalator Displacement), a test based on the displacement of an on/off probe : Thiazole Orange.[2, 3] This method requires only a fluorimeter and a non-modified oligonucleotide. One drawback can be the compatibility of the spectral properties of the tested ligands towards the probe. To circumvent this drawback, we extended the spectroscopic range of the test by using different probes (Hoechst, TOPRO3).[4, 5] A further development of this approach was meant to be an off/on designed test as it could be more sensitive to observe an increase of fluorescence compared to a loss. Recently, we described a fluorescent chemical library of DNA ligands.[6] These styryl dyes are fluorescent in the free state with moderate to good quantum yields and most of them are quenched when bound to quadruplexe sequences. Inspired by this study, we synthesised a new styryl dye named Phen-2,9-Py. This compound is largely inspired by Phen-DC3, a very powerful G4 ligand.[7] We will discuss the scope and limitations of this new tool for G4-FID



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Engineering FRET pairs to study the dynamics of protein conformational changes with a frequency-domain perturbative technique involving temperature oscillations

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We have developed an instrument which enables to measure the rate constants of reaction involving biomolecules using the response of the latter species to small thermal modulations (Fig. 1) [1]. Such kind of technique has already provided valuable information regarding the pairing between oligonucleotides [1,2] and we wish to apply it to the measurement of conformational change kinetics in proteins (upon heating or ligand binding). We have selected the folding/unfolding of the phosphoglycerate kinase enzyme as a starting point since a FRET construct enabling dynamics investigations with a T-jump setup already exist [3]. In the initial configuration a green fluorescent protein, AcGFP1, was used as a donor and a red fluorescent protein, mCherry, as an acceptor. Herein we present how the FRET pair was modified in order to improve the system reliability and its response upon unfolding. In particular, relying on a SNAP-tag conjugated to an organic chromophore and acting as an acceptor has proved valuable.

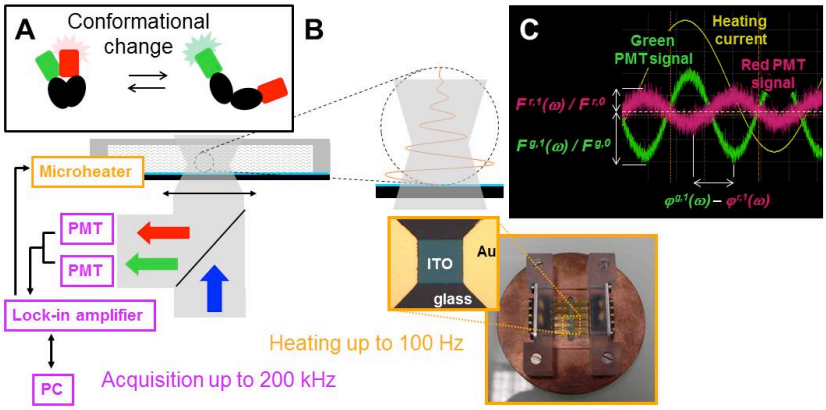

Fig. 1. Principle of the experiment proposed to measure dynamics of conformational changes. (A) The protein of interest is labeled with genetically encoded FRET pair. (B) The reactive system is introduced in a microfluidic chamber heated by an ITO resistor and placed on the stage of an epifluorescence microscope. (C) Injection of a sinusoidal current (—) generates temperature oscillations. In consequence, the chemical system rate constants are also modulated, as well as the species concentrations. The resulting fluorescence oscillations (— and —) are analyzed by lock-in detections, and the variations of the amplitude and phase of the response with the frequency allow the thermokinetic parameters characterizing the investigated chemical system to be extracted.

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Vinyltriphenylamines as fluorescent probes in biological applications

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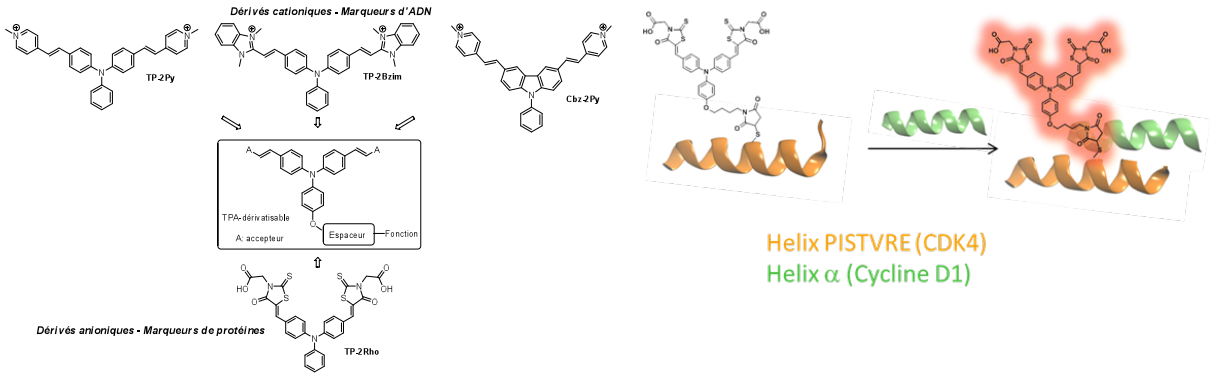
In the field of material chemistry, triphenylamine core is used as donor group to form push-pull system [1]. From the triphenylamine scaffold, we developped biocompatible fluorescent conjugated systems with high charge transfer possessing good two-photon absorption cross-sections. First generation cationic probes bearing pyridinium acceptor groups are efficient on/off fluorescent probes for labelling DNA [2]. Several modifications of the donor core and acceptor groups have been done and increase photophysical properties [3]. By changing their acceptor moities from cationic ones to anionic ones TP-Rho, we were able to switch DNA labelling to protein labelling. These water soluble and cell-permeant π-conjugated molecules known as TP-Rho display optical properties which make them attractive candidates for protein and peptide-based applications.

Here we describe conjugation of this switchable fluorescent dye, TP-2Rho, to peptide and protein derivatives of cyclin-dependent kinase 4 (CDK4) and its application to characterization of the interactions between the catalytic subunit of this kinase, its regulatory partner cyclin D1 and a peptide substrate [5].



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